EVALUATING SOURCES OF OMEGA-3 FATTY ACIDS FOR THEIR BENEFITS ON LATE GESTATION MARES AND NEONATAL FOALS

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Erika A. Snyder

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APPROVED:

Jessica S. Bedore, PhD
Committee Director

Kyle J. Stutts, PhD
Committee Member

Ben Corl, PhD
Committee Member

John B. Pascarella, Ph.D.
Dean and Professor of Biological Sciences
College of Science & Engineering Technology

ABSTRACT

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Maternal supplementation of omega-3 fatty acids during late gestation has been shown to have a variety of benefits on the dam and resulting offspring. However, research in mares and foals is limited. The current study utilized 13 American Quarter Horse mares and their foals, assigned to one of three dietary treatments: a control basal diet (CON, n=5), a basal diet with the addition of a marine algae supplement (ALG, n=5) or a basal diet with the addition of a flaxseed supplement (FLAX, n=3). Mares were placed on treatments 30 days before their expected foaling date and continued through day 5 post- parturition. Samples of plasma were collected from mares prior to beginning supplementation, day 0 post-parturition, day 5 and day 30 post-parturition. Milk samples were collected from mares at day 0, 5 and 30 post-parturition. Plasma samples were collected from foals at birth, day 5 and day 30 post-parturition. Fatty acid compositions of mare plasma, mare milk and foal plasma were determined. Data was analyzed using the MIXED procedure of SAS. Foals born to supplemented mares exhibited increased plasma DHA levels at birth and foals born to ALG mares had greater plasma EPA levels. Samples obtained from foals at birth prior to nursing had the highest DHA content compared to all other samples. No differences were observed in mare plasma or milk. The results of the foal plasma suggest the most efficient avenue for increasing foal DHA levels is through maternal supplementation of omega-3 fatty acids in late gestation and that supplementation for the final 30 days of gestation is sufficient to see changes.

KEY WORDS: Omega-3 fatty acids, DHA, EPA, Marine algae, Flaxseed, Plasma

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CHAPTER I

INTRODUCTION

Essential fatty acids (EFA) are a specific type of polyunsaturated fatty acid (PUFA) composed of a long hydrocarbon chain that contains a double bond between the 3rd and 4th (omega-3) or 6th and 7th (omega 6) carbons from the methyl end. Animals are unable to produce double bonds in these locations, and therefore must consume fats that contain them. This study specifically investigates the omega 3 fatty acids, docosahexaenoic acid (DHA, C22:6n3), eicosapentaenoic acid (EPA, C20:5), and alpha-linolenic acid (ALA, C18:3n3). These PUFA are found in all body tissues but are most abundant in the cellular membranes of nervous, reproductive, and retinal tissues (Arterburn et al., 2006). In cell membranes, DHA incorporates into cell membranes where it promotes more efficient nerve cell signal transmission and resistance to mechanical stress and cellular injury (Stillwell and Wassall, 2003). Docosahexaenoic acid is also used to produce anti-inflammatory compounds of the prostaglandin cascade, and might therefore influence the body's repair response to infection and injury (Ricciotti and Fitzgerald, 2011).

Horses obtain EFA through their native diet as well as from supplemental fat sources. Essential fatty acids can be obtained from forages such as grass hay and pasture, with grasses being abundant in ALA but having nearly undetectable EPA and DHA levels. Additional sources of ALA include seed oils such as those from flaxseed, soybean, and chia seeds, which are comprised of 55%, 8% and 64% ALA, respectively. The predominant omega 3 fatty acid consumed by horses is ALA, which can be elongated to EPA and DHA through an intracellular process. Although DHA can be produced from

ALA, the efficiency of this process is unknown in horses. Some research has investigated efficiency in other species, such as tree swallows which were found to have conversion rates of 3 and 11 percent in the muscle and liver, respectively (Twining et. al, 2018). In humans, conversion rates are estimated between 0.5 and 9 percent when measured in total plasma lipids (Burdge, 2004). Therefore, increasing dietary ALA may not increase the amount of DHA available to nerve cells and the immune system. Direct supplementation of DHA and EPA to horses in the form of fish and microalgae oils may be a more effective way to promote the nerve cell and anti-inflammatory benefits of DHA.

This project investigated DHA, EPA and ALA plasma levels in foals prior to and after consumption of milk from mares supplemented with EFA sources for the last 30 days of gestation. The objective of this project included investigating if foals were obtaining these EFAs through placental transfer during late gestation or through maternal milk after parturition.

CHAPTER II

OBJECTIVE

The objective of this study is to test the hypothesis that foals born to mares supplemented with marine-derived algae during late gestation will have greater plasma DHA concentrations, a shorter time to stand and suckle after birth, and stronger immune responses to colostrum ingestion than foals born to either control mares or mares supplemented with flaxseed-derived ALA.

CHAPTER III

LITERATURE REVIEW

Chemistry of Fats

Dietary fats and oils consist of triglycerides, organic compounds consisting of three fatty acids (FA) bound to a glycerol structure. Triglycerides can appear as either liquid or solid at room temperature depending on the saturation status of their fatty acids. The FA chemical structure is composed of a chain of at least 2 carbon (C) atoms, with a methyl group at the tail (omega end) and a carboxyl group at the head (delta end), which is attached to a glycerol when in triglyceride structure. Fatty acids are then divided into saturated or unsaturated categories. Saturated fatty acids lack double bonds between C atoms and are most often found in dairy products and animal fats (Valenzuela and Morgado, 1999). This lack of double bonds induces a straight and unbending structure that contributes to solidification at room temperature. Unsaturated fatty acids have at least one double bond between C atoms and are most often found in vegetable oils (Sardesai, 1992). The double bonds impart strength allowing for bending of the structure, changing its shape and contribute to the triglyceride being liquid at room temperature. Unsaturated FA can be further divided based on their number of double bonds. Monounsaturated fatty acids have only one double bond while PUFA have two or more double bonds. Fatty acids can also be classified by their carbon chain length, as either short (2-4 C), medium (6-10 C), long (12-18 C), or very long (20 or more C).

Essential Fatty Acid Chemistry

Essential fatty acids are a specific type of PUFA with at least one double bond that is located specifically along the C chain. The production of these specific double

bonds is achieved by desaturase enzymes, each of which desaturate a specific C position. Animals are unable to produce the double bonds in EFA because they lack the desaturase enzymes for the omega 3 and 6 positions (Voet and Voet, 2004). Therefore, these PUFA are essential for inclusion in the diet. Animals are able to consume ALA and elongate the delta end in order to produce DHA, however, the conversion rates vary with species and are still largely unknown (Plourde and Cunnane, 2007). There are two families of EFA, omega 3 and omega 6.

Omega 6 Fatty Acids (LA & AA) structures

Omega 6 EFA are named as such due to the double bond in their structure located at the 6^{th} C atom from the omega tail. This class of fatty acids includes linoleic acid (LA; C18:2n-6) and arachidonic acid (AA; C20:4n-6). Animals are capable of metabolizing LA into AA through chain lengthening, with AA being the active form.

Omega 3 Fatty Acids (ALA, EPA & DHA) structures

Omega 3 EFA are also named for the location of their double bond, with the first double bond in their structure being at the 3^{rd} C atom from the tail. This class of PUFA includes ALA (C18:3n-3), EPA (C20:5n-3), and DHA (C22:6n-3). Animals are capable of metabolizing ALA into EPA and DHA through chain lengthening.

Biological Functions of Essential Fatty Acids

Docosahexaenoic acid is found all throughout the body, but most importantly in the cellular membranes of nervous, reproductive and retinal tissues (Arterburn et al., 2006). Docosahexaenoic acid is the most abundant omega-3 fatty acid in the brain and retina as it comprises 40% of the PUFAs in the brain and 60% of the PUFAs in the retina

(Davis-Bruno and Tassinari, 2011). Five percent of the weight of a neuron's plasma membrane is composed of DHA. Docosahexaenoic acid interacts within cell membranes, incorporating into the membrane phospholipids to alter shape, fluidity, elasticity, and permeability to increase the efficiency of nerve cell communication (Stillwell and Wassall, 2003).

Nerve Cell Transmission

Docosahexaenoic acid has been shown to improve cognition by improving nerve cell transmission. One specific function is modulating the carrier-mediated transport of choline, glycine, and taurine, which are precursors to the neurotransmitters that help to produce cognitive and motor functions (Nelson and Cox, 2005). Docosahexaenoic acid is essential for the growth and functional development of the brain in infants and is required for the maintenance of normal brain function in adults. The inclusion of plentiful DHA in the diet improves learning ability, whereas deficiencies of DHA are associated with deficits in learning (Horrocks and Yeo, 1999). Phosphatidylserine (PS) is a phospholipid component of the cell membrane that controls apoptosis, and low DHA levels in the neuronal membranes lower neural cell PS concentrations, therefore increasing neural cell death (Kim, Akbar and Kim, 2001). DHA levels are found to be reduced in the brain tissue of severely depressed patients leading researchers to believe that due to their anti-inflammatory properties, EPA and DHA can be used in the treatment of depression (Horrocks and Yeo, 1999).

Extensive research in humans has suggested that DHA and EPA supplementation can aid in the prevention of heart disease, cancer, diabetes and autoimmune disorders. O’Connor et al. (2007), hypothesize that this is due to the FA’s ability to increase

vascular compliance and red blood cell deformability, benefits due to plasma membrane fluidity, as well as enhancing insulin sensitivity, which may be more closely related to the anti-inflammatory actions of DHA. DHA has been accepted as necessary in developmental diets for visual and cognitive development in humans and further benefits continue to be researched.

Inflammation: Eicosanoids and Docosanoids

The EFA, EPA and DHA, are also precursors of eicosanoids and docosanoids, compounds that act as signaling molecules for various immunological processes. Eicosanoids that are derived from omega 6 EFA such as prostaglandins and thromboxanes are an integral part of inflammatory and immune responses as well as smooth muscle contractions (Ricciotti and Fitzgerald, 2011). Alternatively, eicosanoids derived from omega 3 EFA such as resolvins and protectins are signaling molecules that are involved in promoting normal cell function after inflammation from tissue injuries by orchestrating the timely resolution of the inflammation (Kohli and Levy, 2009).

Eicosanoids that are produced from the metabolism of AA occur through two distinct pathways. The cyclooxygenase (COX) pathway produces prostaglandins (PG) and thromboxanes (TX) while the lipoxygenase (LOX) pathway produces leukotrienes (LT) and hydroperoxytetraenoic acid and hydroxyeicosatetraenoic acid. Leukotrienes are inflammatory mediators that regulate immune response by triggering contractions in the smooth muscle (Salmon and Higgs, 1987). Specific metabolites and their functions include prostaglandin F2 alpha (PGF2a) that induces uterine and smooth muscle contractions and thromboxane A2 (TXA2), which is a potent vasoconstrictor and induces clotting during tissue injury.

Eicosanoids that are produced from the metabolism of EPA occur through the same COX and LOX pathways, however, have different potency and function. Eicosapentaenoic acid that is metabolized through the COX pathway produces a different type of PG and TX, and similarly, EPA metabolized through the LOX pathway produces a different series of LT. An important eicosanoid derived from EPA is prostaglandin E3 (PGE3) which has vasodilator and anti-inflammatory activities. Arachidonic acid and EPA compete for enzyme availability in the COX and LOX pathways, therefore, dietary intake and supply can regulate the types of eicosanoids being produced and ultimately the inflammation/anti-inflammation balance (King et al., 2008).

Essential Fats in the Equine Diet

Horses originated as purely grazers, with their diet made up entirely of fresh grasses found in their habitats. This type of diet allows the horse to consume an approximate 3:1 ratio of omega 3:6 FA. When horses became domesticated and humans began to feed them grains to increase energy, their diet significantly changed, and the FA ratio with it. The current approximate ratio is inverted to 1:10. This ratio can be restored through the supplementation of FA to the equine diet.

Ingredient Contribution of Fat into Diet

Horses obtain their required energy through their diet. This can be in the form of carbohydrates, protein, or fats. While carbohydrates are often used more in the diets, fats produce 2.25 the amount of energy as carbohydrates or protein. The energy requirement of the horse depends on its current stage of life. Factors such as age, size, workload, location and pregnancy status play a large role in determining the energy requirement. Fats are also important to include in the diet to be able to absorb fat-soluble vitamins and

to provide the horse with essential fatty acids. The National Research Council (NRC) has developed recommended nutrient requirement levels depending on the status of the horse.

Digestion of Fats

Digestion and metabolism of fat in the horse involves a complex series of biological processes that involve a variety of body systems. While human fat digestion begins in the mouth with lingual lipase, equine saliva has limited enzymatic activity and only serves to lubricate food. In equines, true fat digestion begins in the stomach where FA are hydrolyzed from triglycerides by gastric lipase. The majority of the digestion occurs in the small intestine, where bile secreted by the liver into the duodenum acts to emulsify the fat present. Utilizing pancreatic lipase, the hydrolysis of FA from the triglyceride produces two free FA and one 2-monoacyl-sn-glycerol. Short and medium chain free FA are absorbed by passive diffusion into the enterocyte where they enter the portal vein and travel to the liver. Long chain free FA must combine with lysophospholipids and bile salts in the lumen to create micelles. The micelles are then absorbed by the enterocytes by FA transporters. Within the enterocyte, triglycerides are reformed and combined with cholesterol esters and apolipoprotein B to form chylomicrons. Chylomicrons allow the hydrophobic fats to travel into the lymphatic system where they enter the vena cava via the thoracic duct. Once in the blood, chylomicrons react with lipoprotein lipase to release the FA for uptake by tissues and adipocytes.

De novo synthesis of FA in the equine is accomplished by the liver, however not all FA can be synthesized. While mammals are able to insert the double bond at the 9^{th} carbon from the carboxyl (delta) end of the FA, they lack the desaturase enzyme needed

to insert double bonds further (Wathes et al., 2007). This prevents the synthesis of linoleic acid (LA; C18:2n-2) and alpha-linolenic acid (ALA; C18:3n-3) in mammals, making them essential FA that must be provided in the diet. Once consumed, animals can convert LA and ALA to longer chain PUFA through series of elongation and desaturation steps. The LA can become arachidonic acid (ARA; C20:4n-6) and ALA can produce eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3).

Unlike mammals, plants have the necessary desaturase enzymes capable of inserting double bonds into the 12^{th} and 15^{th} carbon positions to create omega-3 and omega-6 FA. Microorganisms are able to desaturate at the 6^{th} carbon of ALA to produce stearidonic acid, which is then elongated to produce eicosatetraenoic acid. That is then desaturated at the 5^{th} carbon to produce EPA which is elongated to produce DPA. DPA is then desaturated at the 4^{th} carbon to produce DHA.

Humans and horses must go through a series of conversions. The resulting EPA is twice elongated (24:5n-3 to 24:6n-3) then shortened to DHA (22:6n-3) via beta oxidation in a pathway otherwise known as “Sprecher’s Shunt.” (Sprecher and Chen, 1999). In humans, research has shown a higher conversion of ALA to DHA in women than in men (Burdge and Wootton, 2002), but it is unknown if gender effects occur in horses.

Sources of Omega Fatty Acids in Equine Diet

Equine diets provide horses with their essential n-6 FA, LA through cereal grains, oilseed meals, forages and oils. These diets are also often supplemented with vegetable oils and other fats in order to increase the energy density of the diet. Oils such as sunflower, safflower, peanut, soy and corn are high in LA making them popular for equine diets. Most forages common in equine diets such as timothy, bermudagrass and

bahiagrass, also contain LA. LA can be desaturated and elongated to produce dihomo-gamma-linolenic acid (DGLA) and the previously mentioned ARA which are both important precursors to eicosanoids. ARA is also one of the two major PUFA found in the neuron-dense grey matter of the brain (Lauritzen and Carlson, 2011). Past research has found that supplementation of n-6 FA to the equine diet results in multiple physiological responses related to exercise, athletic performance and behavior (Woodward et. al, 2007).

The other essential equine FA, ALA is an n-3 FA. Most of the ALA in the equine diet is derived from forages but it is at very low concentrations in regards to percent of total fatty acids. ALA is found mainly in seed oils such as flaxseed (linseed, 55%), soybeans (8%), and chia seeds which have up to 64% ALA as a percent of oil. ALA serves as the precursor for synthesis of the n-3 long chain PUFA, EPA and DHA.

While DHA can be synthesized from ALA, it can also be obtained directly from maternal milk, fish oil, or algae oil. Most of the DHA in fish and multi-cellular organisms with access to cold-water oceanic foods originates from photosynthetic and heterotrophic microalgae and becomes increasingly concentrated in organisms the further they are up the food chain. DHA can also be commercially manufactured from microalgae. In strict herbivores, DHA is manufactured internally from α-linolenic acid, a shorter omega-3 fatty acid. To be effective, DHA and EPA need to be supplemented to the equine diet in the form of fish and microalgae oils.

Elongation and Competition

In humans, the ALA to DHA conversion rate is thought to be < 1% (Arterburn et al., 2006), and the conversion rate in horses is thought to be similar (Hess et al., 2012).

Past research has shown no impact on the proportion of DHA in the blood when supplemented with flaxseed (ALA) (Hess et al., 2012; Vineyard et al., 2010). Therefore, it seems that the only way to increase DHA within equine blood circulation is to supplement directly with a DHA source such as fish oil or marine algae (Hall et al., 2004; King et al., 2008; Vineyard et al., 2010).

As discussed earlier, LA and ALA can be used as precursors to synthesize various long chain PUFA. This extensive conversion process uses the delta-6 and delta-5 desaturases for both n-6 and n-3 production. The limited availability of these enzymes causes competition between LA and ALA for use of the two pathways. The resulting ARA, EPA and DHA levels are therefore impacted by this competition.

Proper n-6 to n-3 Ratio

Omega-3's have been shown to reduce inflammation while omega-6's have been shown to promote inflammation, making it vital to improve the ratio of n-3:n-6. Inflammation is vital to mediate tissue repair, so omega-6 fatty acids should still be incorporated into the diet. Human research has found that the average American citizen's diet reflects a 17:1 n-6 to n-3 ratio (Simopoulos, 2008). However, further research suggests that the ideal n-6 to n-3 ratio for humans is around 4:1 (Davis-Bruno and Tassinari, 2011). The ideal ratio for equines has yet to be established.

Equine Reproduction

The industry and terminology

Horses are considered "long day breeders," meaning they will come into estrous as the length of the days increases in the spring. Mares will have multiple estrous cycles

throughout the spring and summer as they are seasonally polyestrous. In the equine industry, mares can be bred by live cover or artificial insemination. Live cover involves both the mare and stallion being physically present and involves the full process of vaginal intercourse. Live cover can be done with human intervention or with no outside interference such as pasture breeding. Artificial insemination involves the insertion of previously collected stallion semen into the mare's reproductive tract. Artificial insemination allows the broadened use of high quality genetics as well as lowers the risk of injury or disease to both the animals and humans involved.

The normal range for the equine gestation is 335 to 360 days. This results in mares giving birth, or foaling, during the late winter to early summer. As mares approach parturition their body will begin to alter in preparation. Signs begin up to two weeks before foaling and can include accumulating milk in the udder. Approximately 24 hours before foaling the muscles around the tail head and vulva will relax and dried milk may accumulate on the ends of the teats, otherwise known as waxing. The process of parturition is regarded in three stages. Stage one begins when the mare begins to show to be restless, often laying down and standing repeatedly. Stage one ends when the amniotic sac protrudes from the vulva and breaks, releasing the amniotic fluid. Stage two involves the appearance of the foal, starting with one front hoof soon followed by the next, followed by the nose showing the foal is in the sternal position, and quickly followed by the remainder of the body. Stage three is the expulsion of the afterbirth and placenta within one to three hours after emergence of the foal. Retention of the placenta past this time is considered a retained placenta and should be treated as an emergency as retention can cause infection, illness and infertility.

Once the foal has been born, the mare will clean off the amniotic fluids and remaining birth sac from the foal. The foal will immediately begin to attempt to stand and a successful attempt can take up to an hour but more often is within 15 minutes. Once the foal has successfully stood and walked, it will attempt to nurse from the mare. This process may take a few hours but it is important for the foal to nurse in the first twelve hours of life to receive the colostrum crucial to its immune system. Foals should also pass the meconium, or first bowel movement, shortly after successful nursing. IgG levels should be checked at twelve hours of life to ensure proper colostrum intake.

Broodmare management

Broodmare nutrition is vital to ensuring the success of a breeding program when the goal is to produce live, healthy foals. Good body condition is essential to improving reproductive efficiency as it can affect conception rates, anovulatory length and number of cycles per conception. To improve body condition, fat is often added to the equine diet. Cattle producers often add fat to the diet of their herd in order to increase energy content and this has shown to also have positive impacts on reproductive health (Gulliver et al., 2012).

Nutritional Assessments

A Body Condition Score (BCS) assessment can help to determine the nutritional status of a horse from an external inspection. The Henneke System (1983) uses a 9-point scale, with 1 being poor and 9 being extremely fat. The accepted ideal range is from a 4 to a 6, depending on the horse's individual needs. The score is determined by an overall visual appraisal as well as a palpation of the 6 major far cover points.

Another equine nutritional assessment is the rump fat thickness measurement. A point is determined at approximately 5 cm lateral from the midline at the midpoint of the pelvic bone and the fat thickness is measured using b-sound ultrasonography. This measurement can then be used in an equation to determine the animal's body fat percentage. The equation is as follows (Westervelt et al., 1976):

% body fat = 8.64 + {4.7 X (rump fat in centimeters)}

PUFA Supplementation During Gestation

Gestation Length

While cattle research has shown the specific FA influence on reproductive mechanisms, there is limited research done on the reproductive efficiency of mares when supplemented with specific fatty acids.

Human trials suggest that third trimester diets high in n-3 FA may increase the length of gestation (Gulliver et al., 2012). It is hypothesized that the previously described competition for delta-6 desaturase and COX pathway enzymes increases series 1 and 3 PG while decreasing the series 2 PG that produces stronger uterine contractions (Duvaux-Ponter et al., 2004). Pickard et al. (2008) observed a tendency for longer gestation in ewes supplemented with DHA in the form of algae oil when compared to a control diet. Capper et al. (2006) also observed that when ewes were supplemented with fish oil, gestation increased significantly by 2 d compared to ewes supplemented with palm oil. However, no treatment difference was observed in gestation length (Mattos et al., 2004) or rate of placental expulsion (Kemp et al., 1998) in dairy cows that were supplemented with diets differing in linoleic and linolenic acid contents. When investigated in mares,

Duvaux-Ponter et al. (2004) reported that the gestation length was not affected by diet when pregnant mares were supplemented with either linseed (ALA) or rapeseed (LA) for 1.5 months prior to their expected foaling date.

Transfer to the Fetus

The last trimester of pregnancy in mares requires an increase in energy, protein, calcium and phosphorus due to the significant increase in fetal growth. During gestation, the placenta provides maternal nutrients to the fetus, gas exchange, hormone secretions, and the elimination of fetal wastes through simple or facilitated diffusion and active transport. The mare has a diffuse epitheliochorial placenta with clusters of microcotyledons that are formed from numerous chorionic villi connecting the fetal and the maternal placental interface (Senger, 1997). In humans, maternal transfer of ALA and LA to the fetus is influenced by maternal PUFA intake, functionality of the placenta, and ability of DHA mobilization/uptake by fatty acid binding proteins and fatty acid transporters in the maternal placenta side (Lauritzen and Carlson, 2011).

In humans, there is a higher concentration of FA in plasma during the last trimester of pregnancy due to a maternal change in lipid metabolism in which fat deposits are catabolically broken down (Herrera, 2002). Triglycerides cannot pass directly through the placenta, but LPL and lipoprotein receptors along with facilitated membrane translocation within the placenta enable FA to transfer into the placenta and ultimately to the fetus (Herrera, 2002). DHA seems to be accumulated more than any other FA in fetal circulation suggesting specificity for DHA transport across the placenta which also contributes to the higher amounts of DHA in fetal adipose tissue after birth (Lauritzen and Carlson, 2011).

It is hypothesized that the equine placenta is permeable to FA and that the equine placenta and/or the foals are able to synthesize the long chain PUFA from the maternally derived essential FA. While it is still uncertain if the placenta or foal possesses the ability to convert ALA to DHA, a study showed that umbilical vein plasma had concentrations of long-chain PUFA derived from LA and ALA, which were not seen in the maternal plasma suggesting placental or fetal conversion (Stammers et al., 1991). How this occurs is still unknown.

Post foaling behavior

The instinctual behavior of neonates has also been shown to be altered by n-3 supplementation in other species. In lambs, the latency of time to stand was decreased for lambs born to ewes fed algae oil compared to control diets (Pickard et al., 2008). Similarly, the latency to suckle in newborn lambs was decreased when ewes were fed a fish oil supplemented diet (Capper et al., 2006). While the actual cause behind the behavioral change is unknown, when lambs were sacrificed, a lower n-6 to n-3 ratio in the brain tissue was noted in supplemented lambs (Capper et al., 2006).

Rebreeding effectiveness

N-3 supplementation has also been shown to have positive effects after parturition in preparation for the next breeding. Rebreeding of mares and ensuring a successful pregnancy on the first attempt is vital to optimizing production and performance. This is often done by rebreeding on foal heat, the first follicular cycle usually occurring six to eight days after parturition. DHA supplementation has been shown to improve uterine involution, a critical process to prepare the uterus for the next embryo (Adkin, 2013).

Ovarian artery blood flow has also been improved with n-3 supplementation, thus increasing blood flow to the dominant follicle before foal heat. Improved vascularitiy of follicles prior to ovulation has been shown to improve fertility in mares (Silva et al., 2006). It is thought that oocyte viability may be linked to follicular size and therefore overall fertility (Morel et al., 2010). In cattle supplemented with flaxseed oil (n-3 FA), the average diameter of the ovulatory follicle was reported to be significantly larger than those cattle supplemented with sunflower seed oil (n-6 FA) (Ambrose et al., 2006). This study also showed improvements in pregnancy rates and lowered pregnancy losses in n-3 supplemented cows. In contrast, Gulliver et al. (2012), found that n-6 FA supplementation in cattle resulted in lower pregnancy rates. It is hypothesized that diets high in n-6 FA increase the PGF2a concentration and may lower fertility in cattle due to a loss of a functioning corpus luteum (Gulliver et al., 2012). Currently this has not been investigated or confirmed in mares.

N-3 supplementation has been beneficial in improving breeding success in mares for both insemination and live cover techniques. For example, omega-3 supplementation has been shown to inhibit the production of cytokines, chemicals critical for inflammation response. A 2014 study by Brendemuehl et al., using omega-3 supplements demonstrated a reduced inflammatory response in mares bred with frozen semen. For live cover breedings, omega-3 supplemented mares have been shown to be less likely to have uterine fluid (23%) compared to non-supplemented mares (50%) (Brendenmuehl, Kopp and Altman, 2014).

Passive Immunity Transfer to Foals

When foals are first born they lack the proper antibodies for full immunity to new pathogens. Therefore, foals must receive these antibodies through the mare's first milk, or colostrum. Mare colostrum consists of 3 important immunoglobulins: immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA). As the mare approaches parturition, colostrum is produced in the mammary tissue with the highest concentrations of IgG and IgM available to the foal immediately after birth. The concentration and availability of these immunoglobulins rapidly declines each hour postpartum. By hour 12 to 18 postpartum, IgG concentrations in mare mammary secretions are only 5 to 25% compared to the levels immediately after foaling, and by 8 to 16 h postpartum are too low to provide any significant immunity (Lewis, 1995). Newborn foals must ingest and absorb colostrum immunoglobulins to reduce risk of infection as they are without circulating immunoglobulins at birth (Lewis, 1995). Immunoglobulins cannot cross the maternal-fetal placenta interface and thus, the foal must receive the mare's immunoglobulin resistance against disease via colostrum until the foal develops an immune system of its own. Absorption of the immunoglobulins occurs through enterocyte cells that line the lumen of the small intestines (McClure et al., 2001). IgA is special as it is not absorbed by the small intestines, but instead provides the gastrointestinal tract with protection against infection and, as opposed to IgG and IgM, its concentration increases postpartum (Lewis, 1995).

N-3 supplementation has been shown to have beneficial effects on enterocyte function and immunoglobulin absorption. Some research suggests that an enterocyte has

better fluidity when the plasma membrane is composed more of PUFA than of saturated fatty acids (Duvaux-Ponter et al., 2004). This may allow for more immunoglobulins to pass into PUFA rich enterocytes within the small intestine of the newborn foal. However, no treatment differences were observed in IgG levels measured in foal plasma when pregnant mares were supplemented with either an ALA (linseed) or LA (rapeseed) during early gestation and lactation (Duvaux-Ponter et al., 2004). While the researchers did not directly investigate if DHA concentrations in the dams milk, or foal plasma, were altered by either treatment, they suggest that the conversions of ALA and LA to long-chain PUFA may have been preferentially assimilated into brain tissue instead of the intestinal membranes. DHA supplementation has been shown in sheep to increase IgG content in colostrum (Keithly, 2010). Little literature exists on the effects of foal passive transfer when mares are supplemented with DHA during gestation and lactation. Stelzleni et al. (2006) reported no effects on immunoglobulin concentrations in mare colostrum or foal serum when pregnant and lactating mares received diets containing flaxseed, fish oil, or no supplementation.

Mare Lactation and the Effects of PUFA Supplementation

Compared to cow or human milk, mare milk is relatively low in fat. Initial secretion of colostrum only contains 0.7% fat and only increases to 1.8% fat during the first month of lactation (Lewis, 1995). Mare milk fat content then continues to slowly decrease after the first month of lactation. According to Lewis (1995), if a mare is adequately fed, her milk will provide the required nutrients needed by the foal for up to 2 months. Mare milk has an abundant amount of short and medium chain fatty acids and PUFAs, with the largest proportion being palmitic acid (C16:0) at 12 to 28% of the total

FA and LA and ALA making up 5 to 20% and 12%, respectively (Doreau and Martuzzi, 2006). Longer chain derivatives of LA and ALA, such as EPA and DHA have been found in very small quantities (less than 0.05 % of total FA) in mare milk (Gettinger, 2010). Dietary consumption of ALA can affect the concentration of ALA in mare milk. For example, consumption of a diet rich in fresh grasses has been shown to increase the proportion of ALA in milk fat to 15 to 25% of total FA as compared to 10% of total FA when fed a winter diet devoid of fresh grasses (Doreau and Martuzzi, 2006). LA levels can also be altered as Zeyner et al. (1996) found higher concentrations of LA in milk when supplementing lactating mares with soybean oil. These results seem to suggest that the FA concentration of mare milk could be influenced by n-3 supplementation, particularly DHA.

Ideal Amount of DHA Supplementation During Mare Gestation

While the ideal amount of DHA supplementation for the equine diet is still under investigation, human supplementation rates of 1.62 g DHA per day have shown a significant increase in DHA fatty acid profiles (Conquer and Holub, 1996). So far, equine nutritionists have adapted this effective level and compensated for a horse's much larger size. In 2007, Woodward et al. fed horses 5.95 g of long chain n-3 PUFA containing 1.41 g of EPA and 3.18 g of DHA daily and observed a significant increase in plasma DHA. In 2013, Adkin was able to supplement mares with only 2 g/d of DHA and still observed an increased concentration of DHA in mare plasma, umbilical cord plasma, foal plasma, mare RBC and mare milk (Adkin, 2013). However, research has indicated that levels are quickly discontinued after supplementation is ceased (King, 2007).

CHAPTER IV

MATERIALS AND METHODS

Prior to the start of this study, the Sam Houston State University Institutional Animal Care and Use Committee approved all procedures involving animals.

Animals

Study 1

Thirteen pregnant American Quarter Horse mares and their resultant foals were used for this study. Mares were sourced from local breeders. Mares ranged in age from 5-22 yr and were at 310 days of gestation when treatments commenced.

Study 2

Surplus blood and milk samples from American Quarter Horse mares and foals were obtained from local equine breeding farms in order to investigate normal ranges of plasma lipid concentrations in broodmares receiving industry standard diets. These samples were obtained from routine postpartum care protocols. These mares were housed in various locations as well as were on varying diets according to their management.

Study Design

Study 1

Mares were assigned to 1 of 3 treatments: unsupplemented control (CON; n=5), flaxseed (FLAX; n=3), or algae (ALG; n=5). The number of horses per treatment was determined based on a power analysis using data from previous research investigating plasma DHA concentrations in horses fed an algae-based supplement. Two horses are

missing from the FLAX treatment due to the COVID-19 pandemic, which shut down research operations prior to trial completion.

Mares arrived at SHSU approximately 2-5 days prior to beginning treatments. Mares began supplementation 30 days prior to their expected foaling date (EFD) and continued for 5 days after parturition. Expected foaling dates ranged from January to June. For this reason, mares were sequentially assigned to treatments in order to minimize the effect of season, day length, and temperature.

The study began Mid-January 2019 (n = 11; 4 CON, 4 ALG, 3 FLAX) and ended late March 2020 (n=2; 1 CON, 1 ALG). All mares were housed at the Sam Houston State University Agriculture Center (Latitude: 30.705592, -95.544408) for the duration of supplementation and through 5 d post foaling. All mares were housed individually in foaling stalls (3.66 m x 7.32 m) and allowed 1 hour of turnout time in a covered arena, daily. Five days after parturition, mares and foals were returned to their respective owners and supplementation ended. Routine vaccinations, deworming, and farrier schedules were maintained throughout the study.

Study 2

Mares and their resulting foals remained at their place of ownership and remained on normal diets and management practices. Many of the mares consumed diets including alfalfa hay and a pelleted ration designed for mares and foals which contained higher than 14% crude protein. Blood and milk samples were obtained from these mares and their resulting foals within 10-14 h postpartum, per normal husbandry procedures evaluating foal health and colostrum quality.

Treatments and Diets for Study 1

The basal ration for all 3 treatment groups included Coastal Bermudagrass hay, fed at 2% of BW, daily (Table 1). A grain-based concentrate (Calf Creep 14%, Producer's Cooperative, Bryan, TX; Table 1) was fed at 0.6% BW, daily. Hay and concentrate were split into two equal meals, fed at 0600 h and 1800 h. Fresh water was available ad libitum. The diet was formulated in accordance with the National Research Council's recommendations for mares that are pregnant or lactating (NRC, 2007). Foals also had access to their dam's concentrate and supplement during twice daily feedings. Mares receiving a treatment had their supplement top dressed onto their individual basal ration. The ALG treatment consisted of an algal source of DHA (Probiotech, Saint-Hyacinthe (Quebec) Canada; ALG), while FLAX consisted of a flax-based supplement (Smart & Simple Flax, SmartPak, Plymouth, Massachusetts; FLAX). Supplementation of mares began 30 d before EFD (d 310 gestation) and continued until d 5 post-partum (Table 1). The ALG and FLAX supplements were fed at a rate of 242.4 g and 190 g per day, respectively (120 mg/kg BW (As-fed basis)), which resulted in 40 g of DHA + EPA provided to the ALG treatment and 40 g of ALA provided to the FLAX treatment, daily. Supplements were hand-mixed into the grain concentrate immediately prior to feeding. Supplements were stored at 4° C until weighed and fed. The nutrient compositions of the grain-based concentrate, ALG and FLAX supplements, and Coastal Bermudagrass hay are listed in Table 1.

Table 1. Nutrient composition of three diets designed to differ in fatty acid content fed to late gestation mares for a minimum of 30 days.

			Treatment Supplement	
Nutrient (DM basis)	Hay	Pellets	ALG	FLAX
DE, MCal/kg	0.82	1.43	2.85	2.16
Crude protein	9.5	22.8	0.5	24.1
ADF	39.1	11.0	20.6	17.1
NDF	72.2	25.2	36.8	24.2
Fat	2.0	3.0	72.1	32.3
Ash	7.35	6.54	7.73	4.12
FA as % of Total FA				
C16:0	27.79	21.23	1.62	0.52
C16:1	19.36	14.03	22.48	5.34
C18:0	0	0	1.13	0
C18:1	10.32	18.22	0	0
C18:2 n6	7.66	8.56	38.19	29.06
C18:3 n3	11.90	2.92	0	11.22
C20:0	0	4.39	2.35	52.61
C20:4 n6	0	1.03	1.55	0.08
C20:5 n3 (EPA)	0	0	0	0.05
C22:6 n3 (DHA)	0	4.54	7.64	0

Sample and Data Collection

Blood and Milk Collection

All blood samples were collected from the jugular vein, into syringes. Blood was then transferred into heparinized, evacuated tubes (Vacutainer, BD). Blood samples were collected from pregnant mares on the day prior to beginning supplementation, and at 1, 5, and 30 d post parturition. Blood was collected from the foal immediately following parturition and prior to suckling, 12 h post parturition, and 5 and 30 days post parturition (Figure 1). Following blood collection, samples were centrifuged for 10 min at 1500 × g and 4°C, after which plasma was harvested and frozen at −80 °C until analysis.

A blood sample was also collected from each foal at 12 h post parturition for Serum IgG concentration. Samples were placed in uncoated, evacuated tubes (Vacutainer, BD) and allowed to clot at room temperature for 2 h. Serum was harvested and stored in the same manner as plasma.

Between 20 and 30 mL of milk was manually collected into 50 mL collection tubes from both teats of the mare at 12 hr postpartum, 5 days after parturition and 30 days after parturition (Figure 1). Milk was individually frozen at −80 °C until analysis.

Fig. 1

Day 309 Gestation	Day 325 Gestation	Post Parturition	12 h Post Parturition	Day 5 Post Parturition	Day 30 Post Parturition
15 mL of blood and 25 mL of milk from mare BCS, Weight, US	Weight	15 mL of blood from mare and foal	25 mL of milk from mare and 10 mL of blood from foal for IgG count BCS, Weight, US	15 mL of blood from mare and foal 25 mL of milk from mare BCS, Weight, US	15 mL of blood from mare and foal 25 mL of milk from mare

Figure 1. Timeline of sample collection for the investigation of the effects of long-chain fatty acid supplementation of late gestation mares and their resulting foals.

Body Condition Scores and Percent Body Fat

Body Condition Scores (BCS) were subjectively appraised by the same researcher prior to starting supplementation, 12 h postpartum and 5 days postpartum. Scores were assigned using the 9-point scale described by Henneke et al. (1983), ranging from BCS 1 (very poor) to BCS 9 (extremely fat).

Rump fat thickness of mares was measured using B-mode ultrasonography prior to starting supplementation, and at 12 h 5 d post parturition. Measurements were taken from the rump at 5 cm lateral from the midline at the midpoint of the pelvic bone (Westervelt et al. 1976). The region was scanned and the position of maximal fat thickness was used as the measured site. Percent fat was estimated from the equations of Westervelt et al. (1976), where: % fat = 8.64 + (4.70 x cm rump fat).

Foal Measurements and Time to Stand and Nurse

Measurements of each foal were taken at 12 h and 5 d postpartum. Measurements included weight, heart girth circumference, wither height, hip height, body length and cannon circumference.

Time to stand was measured by starting a timer when parturition was complete and stopping time when the foal stood for a continuous 10 s. Time to nurse was measured by starting at the time that parturition was complete and ending when the foal had successfully latched onto the mare's teat and was able to suckle.

Sample Analysis

Lipids were extracted from 3 mL plasma, dried and ground feed samples, milk fat cakes (following centrifugation) using 3:2 hexane-isopropanol as described by Corl et al. (2002).

Milk Fat Extraction

Lipids were extracted from milk fat cakes using 3:2 hexane-isopropanol (HIP) as described by Corl et al. (Corl et al., 2002). Mare milk samples were thawed at room temperature and then weighed into centrifuge tubes to 40 g total weight. Samples were centrifuged at 5,300 x g for 30 min at 8° C. Fat cakes were then weighed out and 300-350 mg placed in a 16x150 screw top test tube. Eighteen mL hexane-isopropanol was added per g of fat cake and then vortexed for 30 s. Twelve mL of sodium sulfate solution per g of fat cake was then added and once again vortexed for 60 s. Samples were then centrifuged at 1000 x g for 5 min. The upper phase was transferred using a Pasteur pipette to a test tube containing 1 g of Na2SO4 and then purged with nitrogen. After 30

min of rest, the solvent was transferred to a fresh 16x100 screw top test tube and loaded onto an N-Evap with dry bath at 40° C. The solvent was then evaporated under a stream of nitrogen. Forty milligrams of the lipid was weighed out into a 16x100 screw top test tube and purged with nitrogen and stored at -20° C until methylation.

Milk Fat Methylation

Previously stored samples were warmed to room temperature and 2 mL of hexane and 40 µL of methyl acetate was added. Samples were then vortexed for 30 s and 40 µL of methylation reagent (0.4 mL sodium methoxide and 1.75 mL methanol) was added and then vortexed again for 2 min. After 8 minutes of rest, 60 µL of termination reagent (1 g oxalic acid dissolved in 30 mL diethyl ether) was added and then vortexed for 30 s. A small scoop of calcium chloride was added to the vial and the vial rested for 1 h. Samples were centrifuged at 2000 x g for 5 min and then the upper phase was transferred to a GC vial (Chouinard and Corneau, 1997).

Plasma Fat Extraction and Methylation

Plasma samples were thawed at room temperature and 3 mL of HIP was added to 2 mL of plasma. Two milliliters of sodium sulphate was added, vortexed, and the upper phase removed. The sample was dried over 1 g of anhydrous sodium sulfate and the solution transferred to a fresh tube and dried under a stream of nitrogen. To this tube, 500 µL of hexane was added as well as 40 µL of methyl acetate. The sample was then vortexed and 40 µL of methylation reagent (0.4 mL sodium methoxide and 1.75 mL methanol) added and then vortexed again. The samples were allowed to react at room temperature for 24 h and then 60 µL of termination reagent (1 g oxalic acid dissolved in

30 mL diethyl ether) was added. A few grains of calcium chloride was added to remove the water and the upper phase was removed for analysis (Perfield et al., 2006).

Feed Lipid Extraction and Methylation

Hay, grain and supplement samples were weighed out to 300 mg and placed into 16x150 screw top test tubes. Then 5.4 mL of HIP was added to each sample and vortexed for 30 s. Fat was then homogenized in HIP in a 12x75 snap cap tube and the homogenate transferred to glass test tube. Three mL of sodium sulphate solution was then added to each sample and vortexed for 30 s twice. Samples were then centrifuged at 1000 x g for 5 min and the upper phase transferred using a Pasteur pipette to a test tube containing 0.5 g Na_2SO_4. Samples were purged with N_2 and let stand for 30 min. The solvent was then transferred to a fresh 16x100 screw top test tube and loaded onto N-Evap with water bath at 40°C. The solvent was then evaporated under a stream of N_2. One mL of toluene and 2 mL methanolic-sulfuric acid were added to the tube containing the extracted lipids and incubated in the heat block overnight at 50°C. One mL of 5% NaCl solution was added to the samples and extracted twice with 2 mL hexane transferring extracts to another test tube. Then 0.8 mL of 2% $KHCO_3$ was added to the extracts and the upper hexane phase removed to a test tube containing 0.5 g Na_2SO_4, and let stand for 30 min. The solvent was then transferred to a new test tube and evaporated under nitrogen. Using 1 mL hexane, the sides of the test tube were washed and the solvent evaporated under nitrogen. Finally,100 µL of hexane were added to the dried extracts and transferred to a GC vial insert using a 5.75” Pasteur pipet for analysis (Corl, 2012).

Analysis

Fatty acid methyl esters from all samples were analyzed by gas chromatography (Agilent GC system 6890N with flame ionization detector using a DB-FastFame 20 m x 0.18 mm i.d. column with 0.2 µm film thickness (Agilent Technologies)) to determine fatty acid composition. Injection port and detector temperatures were 275°C with a flow rate of 0.64 mL/min and was splitless. Initial oven temperature was 150°C and held for 1 min and then there was an increase at 4°C/min to 210°C. Peaks corresponding to specific fatty acids were identified using specific markers (Pure Methyl Ester Standards 68D and 91, Nu-Check Prep Inc.). Although chromatographic analysis will result in the isolation and quantification of numerous FA, data will be analyzed and results reported for the following FA of particular interest: LA, ARA, ALA, EPA, and DHA.

Serum IgG Analysis

Foal serum samples were analyzed for IgG content using a commercially available ELISA test kit (IMMUNO-TEK, ZeptoMetrix, Buffalo, NY). Serum samples were diluted to 1:200,000 in assay diluent for initial testing. 200 µl of standards and 200 µl of samples were pipetted into duplicate wells, covered and then incubated for 30 min at 37° C. The contents of each well were aspirated and the wells washed 4 times with 300 µl of plate wash buffer. Then the plated were blotted dry. 100 µl of provided antibody detector was added to each standard and sample well and then covered and incubated for 30 min at 37° C. The plate was once again washed 4 times and then 100 µl of substrate was added to each well. The plate was incubated for 30 min at room temperature until a blue color developed in wells containing equine IgG. 100 µl of the stop solution was added to each well to obtain a color change of blue to yellow. Optical density of each

sample was measured at 450 nm and the IgG concentration calculated using values provided by test kit.

Statistical Analysis

All statistical analyses were performed using the MIXED procedure of SAS (v.9.4, SAS Inst., Inc., Cary, NC). Fatty acid analysis used repeated measures ANOVA for the effects of time and treatment and the time by treatment interaction. Simple effect differences for time were detected by a Dunnett test which compares all time points back to time 0, and simple effect differences for treatment and the time by treatment interaction were determined using Tukey tests, which makes all possible comparisons. The autoregressive covariance structure for repeated analyses was the lowest AICC. The repeated term was day of study within horse. Growth measurements were evaluated for the effects of treatment and simple effect differences were determined using Tukey Tests.

CHAPTER V

RESULTS

Feed Intake

All mares completed the study and readily consumed the Algae (ALG), Flax (FLAX) and Control (CON) diets with no feed refusals (Table 2).

Table 2. Daily average nutritional intake of mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Nutrient	Nutrient Intake		
	CON	ALG	FLAX
Digestible Energy, Mcal	30.2	28.6	28.7
Crude Protein, kg	1.80	1.71	1.73
Acid Detergent Fiber, kg	4.64	4.40	4.38
Neutral Detergent Fiber, kg	8.74	8.27	8.24
Ether Extract, g	318.4	470.0	360.0
18:2 n6, g	25.3	90.2	41.6
18:3 n3, g	29.0	27.1	34.0
20:5 n3, g	0	0	0.03
22:6 n3, g	4.5	17.4	4.5

Mare and Foal Bodyweight

No treatment differences existed for BW among mares throughout the study ($P > 0.4$; Table 3). Average BW at study commencement (d 310 of gestation) for ALG mares was 518.0 ± 11.4 kg, 518.5 ± 14.7 kg for FLAX mares and 550.7 ± 11.4 kg for CON mares. The average BW at study completion (d 5 postpartum) for ALG mares was 492.60 ± 11.4 kg, FLAX 491.69 ± 14.7 kg and CON 501.21 ± 11.4 kg. Bodyweight was affected by day ($P < 0.001$), but not the time x treatment ($P = 0.33$) interaction, whereby average BW on

day -30 (529.51 ± 8.9 kg) was greater than on day 0 (499.76 ± 8.9 kg; P <0.001), and 5 (495.44 ± 8.9 kg; P= 0.001).

Table 3. Bodyweights of mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition and bodyweights of unsupplemented controls (CON).

Treatment (TRT)	Bodyweight (kg)				*P*-Value		
	Day -30[a]	Day 0[b]	Day 5[b]	SEM	TRT	Time	TRT x Time
CON	550.66	501.67	501.21	11.38			
ALG	518	498.95	492.60	11.38	0.48	<0.001	0.33
FLAX	518.45	493.96	491.69	14.65			

Average foal weights were affected by treatment (P= 0.03) but not the time by treatment interaction (P = 0.6; Table 4). Foals born to CON mares weighed more on average (49.9 ± 2.8 kg) than foals born to FLAX mares (39.3 ± 2.8 kg; P = 0.03), but not foals born to ALG mares (44.5 ± 2.13 kg; P > 0.2). Foal BW was affected by time (P < 0.001); whereby foals were heavier on day 5 (47.9 ± 1.4) than day 0 (41.2 ± 1.4; P < 0.001).

Table 4. Bodyweights (kg) of foals born to mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition and bodyweight of foals from unsupplemented controls (CON).

Treatment (TRT)	Bodyweight (kg)				*P*-Value		
	Day 0	Day 5	SEM	TRT Mean	TRT	Time	TRT x Time
CON	46.87	53.16	2.23	49.9[a]			
ALG	41.45	47.53	2.23	44.5[b]	0.03	<0.001	0.64
FLAX	35.38	43.24	2.88	39.3[c]			

Mare Body Fat Percentage and Body Condition Score

Mare body fat percentage was affected by time ($P < 0.001$) whereby body fat was greater on d 0 (11.48 ± 0.24 %; $P < 0.001$) and 5 (11.54 ± 0.24 %; $P < 0.001$) than d -30 (10.2 ± 0.24 %)(Table 5). No differences in percent body fat were observed between treatments ($P > 0.9$) or the treatment by time interaction ($P > 0.6$). Body condition scores were not affected by time, treatment, or the time by treatment interaction ($P > 0.08$).

Table 5. Body fat percentage and body condition score (BCS) of mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Treatment (TRT)	Morphometric Measurement				*P*-Value		
	Day -30	Day 0	Day 5	SEM	TRT	Time	TRT X Time
	--Body Fat, %--						
CON	10.21	11.39	11.45	0.37			
					0.97	<0.001	0.67
ALG	10.50	11.36	11.40	0.37			
FLAX	9.97	11.67	11.75	0.49			
	--Body Condition Score, unit--						
CON	6.7	6.1	5.9	0.37			
ALG	5.7	5.6	5.6	0.37	0.41	0.08	0.52
FLAX	5.8	5.5	5.6	0.60			

Foal Time to Stand, Time to Nurse and Gestation Length

No treatment difference in time to stand was observed among foals ($P > 0.8$; Table 6). Average time to stand was 32.6 ± 12.09 min for foals born to ALG mares, 42.33 ± 15.61 min for foals born to FLAX mares and 36.40 ± 12.09 min for foals born to CON mares.

Table 6. Time to stand (min) in foals born to mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Treatment	Time to Stand	SEM	*P*-Value
CON	36.40	12.09	
			0.88
ALG	32.60	12.09	
FLAX	42.33	15.61	

No treatment difference in time to nurse was observed among foals ($P > 0.2$; Table 7). Average time to nurse was 61.80 ± 13.24 min for foals born to ALG mares, 98.33 ± 17.10 min for foals born to FLAX mares and 83.80 ± 13.24 min for foals born to CON mares.

Table 7. Time to nurse in foals born to mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Treatment	Time to Nurse	SEM	*P*-Value
CON	83.80	13.24	
			0.25
ALG	61.80	13.24	
FLAX	98.33	17.10	

No treatment difference in length of gestation was observed among mares ($P > 0.4$; Table 8) Average length of gestation was 337.20 ± 2.79 days for ALG mares, 331 ± 3.61 days for FLAX mares and 333.8 ± 2.79 days for CON mares.

Table 8. Gestation length of mares (days) supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Treatment	Length of gestation, days	SEM	*P*-value
CON	333.80	2.79	0.41
ALG	337.20	2.79	
FLAX	331.00	3.61	

Foal Plasma Fatty Acids

Analysis of samples identified numerous fatty acids within the samples. This study focused primarily on the following fatty acids: 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, 18: 3, 20:4, 20:5 and 22:6 (Table 9).

Table 9. Fatty acid content of plasma as a percentage of total fatty acids from foals born to mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Treatment (TRT)	Plasma Fatty Acid, %				*P*-Value		
	Day 0	Day 5	Day 30	SEM	TRT	Time	TRT X Time
	--14:0--				0.03	<0.001	0.25
Day Mean	0.31	2.86**	2.56**	0.21			
CON[a]	0.12	2.70	1.73	0.37			
ALG[b]	0.26	2.99	3.30	0.33			
FLAX[ab]	0.55	2.89	2.63	0.42			
	--16:0--				0.97	0.01	0.33
Day Mean	27.39	22.92*	19.21**	1.85			
CON	30.80	22.04	15.57	3.00			
ALG	26.13	22.96	21.37	2.84			
FLAX	25.26	23.76	20.69	3.67			
	--16:1--				0.44	0.003	0.15
Day Mean	2.25	1.30**	1.52	1.26			
CON	5.05	3.19	3.57	1.99			
ALG	1.24	0.65	0.59	1.98			
FLAX	0.48	0.07	0.40	2.56			
	--18:0--				0.82	0.14	0.45
Day Mean	12.39	8.56	9.98	2.29			
CON	10.75	7.61	10.58	3.75			
ALG	13.01	12.43	10.48	3.53			
FLAX	13.42	5.64	8.88	4.56			
	--18:1--				0.36	0.002	0.36
Day Mean	23.37	11.20**	8.69**	2.78			
CON	20.84	16.73	15.72	4.70			
ALG	25.70	7.11	3.57	4.21			
FLAX	23.57	9.77	6.80	5.44			

(Continued)

Table 9. Fatty acid content of plasma as a percentage of total fatty acids from foals born to mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Treatment (TRT)	Plasma Fatty Acid, %				*P*-Value		
	Day 0	Day 5	Day 30	SEM	TRT	Time	TRT X Time
	--18:2 n6--				0.64	<0.001	0.37
Day Mean	23.09	39.48**	45.99***	4.03			
CON	21.38	40.58	48.45	6.71			
ALG	20.74	45.09	53.12	6.16			
FLAX	27.14	32.78	36.40	7.95			
	--18:3 n3--				0.15	0.006	0.09
Day Mean	0.37	6.77**	7.94**	2.22			
CON	0.40	1.76	2.57	3.55			
ALG	0.17	3.24	4.19	3.41			
FLAX	0.17	1.03	1.01	0.16			
	--20:4 n6--				0.19	0.06	0.06
Day Mean	0	0.11*	0.02	0.03			
CON	0	0	0.03	0.05			
ALG	0	0.04	0.05	0.05			
FLAX	0	0.30	0	0.06			
	--20:5 n3--				0.06	0.13	0.06
Day Mean	0.16	0.21	0.005	0.08			
CON	0	0.01	0.01	0.13			
ALG	0.32	0.63	0	0.12			
FLAX	0.18	0	0	0.16			
	--22:6 n3--				0.03	<0.001	0.06
Day Mean	1.15	0.31***	0.02***	0.12			
CON[a]	0.55	0.19	0.007	0.21			
ALG[b]	1.90	0.56	0.08	0.21			
FLAX[ab]	1.11	0.23	0	0.25			

For 14:0, there was no effect of the day by treatment interaction, however there was an effect of treatment ($P = 0.03$) whereby ALG foals (2.18 ± 0.15 %) had greater 14:0 plasma concentrations than CON (1.52 ± 0.17 %; $P = 0.03$). There was also an effect of day ($P < 0.001$) whereby the percent of 14:0 in plasma was lower on day 0 (0.31 ± 0.21 %) than days 5 (2.86 ± 0.21 %; $P < 0.001$) and 30 (2.56 ± 0.21 %; $P < 0.001$). For 16:0, there was an effect of day ($P = 0.01$) whereby the percent of 16:0 in plasma was higher on day 0 (27.39 ± 1.87 %) than days 5 (22.92 ± 1.86 %; $P = 0.07$) and 30 (19.21 ± 1.81 %; $P = 0.007$). For 16:1, there was an effect of day ($P = 0.003$) whereby the percent of 16:1 in plasma was higher on day 0 (2.25 ± 1.27 %) than day 5 (1.30 ± 1.26 %; $P = 0.002$), but not day 30. There was also a tendency for an effect on day whereby the percent of 16:1 in plasma was lower on day 5 (1.30 ± 1.26 %) than day 30 (1.52 ± 1.26 %; $P = 0.08$). For 18:0 there was no effect of treatment, time, or treatment x time interaction. For 18:1, there was an effect of day ($P = 0.002$) whereby the percentage of 18:1 in plasma was higher on day 0 (23.37 ± 2.78 %) than day 5 (11.20 ± 2.78 %; $P = 0.006$) and higher on day 5 (11.20 ± 2.78 %) than day 30 (8.69 ± 2.69 %; $P = 0.002$). For 18:2 n-6, there was an effect of day ($P < 0.001$) whereby the percentage of 18:2 in plasma was lower on day 0 (23.09 ± 4.05 %) than day 5 (39.48 ± 4.03 %; $P = 0.001$) and lower on day 5 (39.48 ± 4.03 %) than day 30 (45.99 ± 3.93 %; $P < 0.001$). For 18: 3 n-3, there was an effect of day ($P = 0.006$) whereby the percent of 18: 3 in plasma was lower on day 0 (0.37 ± 2.24 %) than day 5 (6.77 ± 2.22 %; $P= 0.005$) and lower on day 5 than day 30 (7.94 ± 2.18 %; $P = 0.009$). For 20:4 n-6, there was a tendency for the effect of treatment by day interaction ($P = 0.06$) whereby the percentage of 20:4 in plasma was greater in foals born to FLAX mares on day 5 than all other samples (0.30 ± 0.06 %, $P < 0.05$). For

EPA (20:5 n-3), there was a tendency for effect of treatment by day interaction ($P = 0.06$) whereby foals born to ALG mares on day 5 (0.63 ± 0.12 %) had greater levels than foals born to CON mares on day 0 ($P = 0.06$), day 5 ($P = 0.08$), and day 30 ($P = 0.05$). For docosahexaenoic acid (DHA, 22:6 n-3), there was an effect of treatment ($P = 0.04$) whereby the percentage of 22:6 in plasma was higher in foals born to ALG mares (0.79 ± 0.11 %) than foals born to CON mares (0.55 ± 0.21 %; $P= 0.02$) but was not different from that of foals born to FLAX mares (1.11 ± 0.25 %; $P= 0.22$). There was also an effect of day ($P < 0.001$) whereby the percentage of 22:6 in plasma was greater on day 0 (1.15 ± 0.12 %) than days 5 (0.31 ± 0.12 %; $P = 0.001$) and 30 (0.02 ± 0.11 units; $P < 0.001$). There was also a tendency for effect of treatment by day interaction ($P = 0.06$) whereby the percentage of 22:6 in plasma was greater for foals born to ALG mares on day 0 (1.90 ± 0.18 %) than in the same foals on days 5 ($P = 0.001$) and 30 ($P = 0.001$), and in foals born to CON mares on days 0 ($P = 0.007$), 5 ($P < 0.001$), 30 ($P < 0.001$) and foals born to FLAX mares on days 5 ($P = 0.002$) and 30 ($P < 0.001$).

Mare Plasma Fatty Acids

Analysis of the mare plasma for FA resulted in no significant differences observed apart from 16:0 and 20:2 ($P > 0.1$; Table 10).

Table 10. Fatty acid content of plasma as a percentage of total fatty acids from mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to parturition or in unsupplemented controls (CON).

Treatment (TRT)	Plasma Fatty Acid, %					*P*-Value		
	Day -30	Day 0	Day 5	Day 30	SEM	TRT	Time	TRT X Time
	--14:0--							
Day Mean	0.90	0.67	0.05	0.27	0.42	0.43	0.32	0.61
CON	2.07	0.93	0.05	0.76	0.58			
ALG	0.24	0.52	0.08	0.02	0.58			
FLAX	0.41	0.57	0.04	0.02	0.76			
	--16:0--					0.09	<0.001	0.41
Day Mean	17.07	21.14**	19.53	18.41	1.29			
CON	15.93	18.14	15.45	14.58	1.91			
ALG	16.54	20.21	18.92	19.15	1.91			
FLAX	18.76	25.08	24.23	21.50	2.47			
	--16:1--					0.50	0.75	0.62
Day Mean	0.97	1.07	1.32	1.41	1.43			
CON	2.87	2.95	3.88	4.23	2.25			
ALG	0.05	0.09	0.07	0	2.25			
FLAX	0	0.16	0	0	2.90			
	--18:0--					0.51	0.27	0.78
Day Mean	17.65	13.52	16.08	15.46	2.25			
CON	17.33	8.94	13.79	15.16	3.53			
ALG	20.96	16.44	18.18	16.74	3.53			
FLAX	14.68	15.18	16.27	14.47	4.56			
	--18:1--					0.33	0.24	0.86
Day Mean	4.58	6.20	4.95	5.03	1.91			
CON	6.81	9.80	9.34	9.70	2.99			
ALG	4.73	4.86	3.58	3.49	2.99			
FLAX	2.19	3.94	1.93	1.92	3.87			

(Continued)

Table 10. Fatty acid content of plasma as a percentage of total fatty acids from mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to parturition or in unsupplemented controls (CON).

Treatment (TRT)	Plasma Fatty Acid, %					*P*-Value		
	Day -30	Day 0	Day 5	Day 30	SEM	TRT	Time	TRT X Time
	--18:2 n6--					0.54	0.82	0.19
Day Mean	51.75	53.58	54.22	54.43	3.09			
CON	43.28	53.49	51.97	49.23	4.84			
ALG	53.07	54.44	55.17	57.07	4.84			
FLAX	58.90	52.83	55.53	56.98	6.25			
	--18:3 n3--					0.46	0.54	0.80
Day Mean	2.85	1.96	1.03	1.67	0.89			
CON	5.17	2.82	0.67	2.39	1.39			
ALG	1.66	1.21	0.77	1.75	1.39			
FLAX	1.73	1.86	1.64	0.87	1.80			
	--20:2 n6--					0.03	0.07	0.40
Day Mean	0.16	0.42	0.29	0.58	0.12			
CON[a]	0.07	0.42	0.08	0.23	0.20			
ALG[a]	0.11	0.12	0.18	0.23	0.20			
FLAX[b]	0.31	0.72	0.62	1.27	0.26			
	--22:6 n3--					0.22	0.57	0.26
Day Mean	0.44	0.10	0.54	0.93	0.41			
CON	1.33	0.26	1.45	0.27	0.65			
ALG	0	0.04	0.17	0	0.65			
FLAX	0	0	0	2.53	0.84			

There was an effect of day for 16:0 ($P < 0.001$) whereby plasma percentages of 16:0 were highest on day 0 (21.14 ± 1.29, $P < 0.001$) when compared to day -30 (17.07 ± 1.29 %). There was an effect of treatment for 20:2 ($P = 0.03$) whereby plasma percentages of 20:2 for FLAX supplemented mares (0.73 ± 0.16 %) were greater than those of ALG supplemented mares (0.16 ± 0.12 %; $P = 0.03$) and CON mares (0.20 ± 0.12 %; $P = 0.05$).

Mare Milk Fatty Acids

Mare milk yielded no observed differences for the effects of treatment ($P > 0.3$; Table 11), with the exception of 20:2 which had a treatment effect ($P = 0.008$) whereby ALG treated mares (0.39 ± 0.1) had lower concentrations of 20:2 in their milk than FLAX treated mares (1.36 ± 0.2; $P < 0.01$) but not CON mares (0.6 ± 0.2; $P = 0.7$). There was also an effect of day for 20:2 ($P = 0.01$) where concentrations on day 5 (1.08 ± 0.2) were greater than on day 0 (0.48 ± 0.1; $P = 0.01$).

Table 11. Fatty acid content of milk as a percentage of total fatty acids from mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Fatty Acid	Day postpartum				*P*-Value		
	D 0	SEM	D 5	SEM	TRT	Time	TRT X Time
14:0					0.73	0.49	0.27
CON	5.79	1.58	7.12	3.16			
ALG	6.67	1.41	5.21	1.57			
FLAX	5.53	1.82	9.46	2.21			
14:1					0.32	0.84	0.14
CON	2.01	2.21	6.82	4.42			
ALG	7.25	1.97	0.45	2.21			
FLAX	0.31	2.55	0.06	3.12			
16:0					0.57	0.75	0.18
CON	22.42	2.16	18.58	4.33			
ALG	20.63	1.93	23.17	2.04			
FLAX	25.35	2.50	23.01	2.77			
C16:1					0.48	0.64	0.67
CON	0.02	0.27	0	0.54			
ALG	0.21	0.24	0.05	0.27			
FLAX	0.77	0.31	0.11	0.38			
18:1					0.86	0.44	0.11
CON	28.21	2.85	24.22	5.71			
ALG	21.74	2.55	27.12	2.70			
FLAX	30.88	3.30	18.09	3.67			
18:2					0.85	0.14	0.30
CON	29.13	3.90	32.18	7.81			
ALG	25.63	3.49	30.83	3.88			
FLAX	20.02	4.50	39.58	5.46			
18:3					0.23	0.19	0.31
CON	4.06	2.18	12.77	4.36			
ALG	3.77	1.95	3.15	2.17			
FLAX	2.74	2.51	4.20	3.07			
20:0					0.55	0.43	0.51
CON	0.08	0.15	0	0.31			
ALG	0.06	0.14	0.40	0.15			
FLAX	0.28	0.18	0.28	0.22			

(Continued)

Table 11. Fatty acid content of milk as a percentage of total fatty acids from mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to expected parturition or in unsupplemented controls (CON).

Fatty Acid	Day Postpartum				*P*-Value		
	D 0	SEM	D 5	SEM	TRT	Time	TRT X Time
20:2	a		b		0.008	0.01	0.19
CON[a]	0.61	0.19	0.59	0.39			
ALG[a]	0.005	0.17	0.78	0.19			
FLAX[b]	0.85	0.22	1.88	0.27			
20:3					0.55	0.79	0.55
CON	0	1.30	0	2.60			
ALG	2.83	1.16	0.02	1.30			
FLAX	0	1.50	0.05	1.84			
20:4					0.13	0.93	0.34
CON	0	0.55	0	1.10			
ALG	0.74	0.49	0.44	0.51			
FLAX	0.25	0.63	1.45	0.69			
20:5					0.83	0.61	0.83
CON	0.03	0.02	0	0.04			
ALG	0.01	0.01	0	0.02			
FLAX	0	0.02	0	0.02			
22:6					0.60	0.75	0.73
CON	0.42	1.75	0	3.50			
ALG	3.67	1.56	0.51	1.75			
FLAX	0.64	2.02	0.07	2.47			

Foal Serum IgG:

No significant differences were observed between treatments for serum IgG concentrations of foals at 12 hours of life (Table 12).

Table 12. Serum IgG concentrations at 12 hours of life of foals born to mares supplemented with either 190 g of flax (FLAX) or 242.4 g of marine algae (ALG) daily for 30 days prior to parturition or in unsupplemented controls (CON).

Treatment	Serum IgG, mg/dL	SEM	P-Value
CON	1502	272	0.572
ALG	1158	304	
FLAX	1682	430	

Industry Foal Plasma and Mare Milk Analysis:

Results of foal plasma and mare milk analysis from the accessory industry survey is located in Table 13.

Table 13. Average fatty acid content as a percentage of total fatty acids of industry foal plasma and industry mare milk at 1 day of age.

Fatty Acid	Fatty Acid, %	
	Foal Plasma (n=20)	Mare Milk (n=29)
14:0	2.32	5.75
14:1	0.14	1.83
16:0	16.48	19.04
16:1	0.25	0.02
18:1	15.92	20.63
18:2	54.96	25.97
18:3	3.56	1.48
20:2	0.74	1.90
20:3	0.92	1.13
20:4	0.34	0.02
20:5	0.03	0.02
22:6	0.18	0.59

CHAPTER VI

DISCUSSION

The primary objectives of this study were to compare mare and foal plasma fatty acid compositions and mare milk fatty acid composition to determine whether late gestation dietary omega-3 fatty acid supplementation would increase the percentage of ALA, EPA, and DHA in plasma and milk. Further objectives were to determine whether the source of omega-3 fatty acids, either algae or flaxseed, would have beneficial effects on neonatal foal growth, time to stand, and time to nurse.

The main finding of this study was that foals from supplemented mares benefitted from increased plasma DHA at birth, and further, that ALG foals had greater plasma EPA at birth. This study's results are consistent with other recent studies indicating that the dietary supplementation of marine-derived omega-3 fatty acids to mares in late gestation and early lactation results in increased plasma DHA and EPA levels in their resulting foals (Hess et al., 2012; Adkin, 2013; Kouba et al., 2019). However, there was no difference in EPA and DHA concentrations at birth between foals born to ALG or FLAX treated mares, so it cannot be said that the algae was more effective than the flax supplement at increasing plasma DHA levels. The current study observed slightly different results than Moallhem and Zachut (2012), who observed almost twice the concentration of plasma DHA in newborn calves from dams supplemented with fish oil when compared to calves from flaxseed oil-supplemented or control diet cows. It is possible that our sample size was too low to detect differences. Original planning included sampling of additional experimental units, however these could not be obtained due to the COVID-19 pandemic. Alternatively, it is possible that fetal tissues effectively

elongate and desaturate precursor fatty acids. Stammers et al. (1991) observed that certain long chain polyunsaturated derivatives of the essential fatty acids found in the umbilical venous plasma phospholipid fraction were not seen in the maternal circulating lipids, but the precursor fatty acids were readily available to both fetal and placental tissues, suggesting they had been elongated and incorporated into phospholipids by fetal and placental tissues.

Interestingly, the samples obtained prior to the foal suckling from the algae supplemented mares had the highest DHA content compared to all other samples. This is consistent with other studies that have also observed DHA levels being highest before nursing (Kouba et al., 2019). This provides encouraging evidence that this transfer of DHA from the mare to foal is made during gestation. Fetal transfer is observed in humans, with fetal erythrocytes playing a role in uptake of DHA and ARA from maternal blood (Ruyle et al., 1990). Further, DHA is preferentially transferred to fetal circulation in humans, with greater uptake than other omega 3 and 6 fatty acids (Haggarty et al., 1997). In mares, Stammers et al. (1991) observed a positive correlation between maternal and umbilical vein plasma free fatty acid concentrations which included substantial amounts of essential fatty acids and their longer chain derivatives, suggesting that the equine placenta is permeable to long chain fatty acids. This previous study paired with the current study's observations support the hypothesis that there is placental EFA transfer to the equine fetus when mares are supplemented during late gestation.

The pattern of DHA levels in neonatal foal plasma suggests that placental transfer may be a more efficient mechanism of DHA absorption than maternal milk supply. Long chain fatty acids represent a low percentage of mare's milk, as indicated through both our day 0

milk samples and the samples obtained from industry mares. As milk is the sole component of a neonatal foal's diet, placental transfer may be more efficient than milk consumption at providing foals with long chain EFA.

While both supplements provided ALA (18: 3), FLAX mares consumed more. Despite this, there was no treatment effect on foal ALA percentages at birth. It is possible this is due to preferential transfer of longer chain fatty acids or elongation of ALA to EPA and DHA. These longer fatty acids, and particularly DHA, are necessary for the proper development of brain tissues, which are still developing at the time of birth, therefore production of DHA in utero is essential. Intriguingly, the pattern of ALA in neonatal plasma over time, opposes that of DHA, with a steady increase through the 30 day post parturition sample collection. Similarly, Kouba et al. (2019) observed that foal plasma ALA increased significantly after birth and stayed elevated through 21 days of life. As time increases it is possible that less of the ALA is being converted into DHA allowing for more accumulation of the fatty acid in the foal plasma. It is also possible that foals began consuming more ALA, as they frequently begin consuming pasture, hay, and their dam's feed. However, it is unlikely that any solid feed consumed will be well digested until several weeks of age when the large colon matures (McKenzie and Geor, 2007). Grass hay and pasture are good sources of ALA, but contain only low amounts of DHA.

While not a target of treatment, we also observed changes in omega-6 fatty acid concentrations, mono-unsaturates, and saturated fatty acids. As for the omega-6 fatty acids, both 18:2 n6 and 20:4 n6 increased from birth, although for 20:4 n6, this finding may have been primarily driven by the high percentage in FLAX foals on day 5. Duvaux-Ponter et al. (2004), observed that 18:2 and 18: 3 levels in foal increased after

suckling, whereas in contrast levels of 20:3 and 20:4 were highest at birth and decreased after suckling. As observed from our industry mares and foals, 18:2 n6 is the most abundant fatty acid in foal plasma and mare milk. Similarly, Kouba et al. (2019) also observed that LA (18:2) was the most abundant fatty acid in foal plasma and mare milk across treatments and time. The reason for 18:2 having the highest concentrations could possibly be related to the fact that LA is the precursor to ARA (20:4) which is important for synthesizing a variety of hormones that are vital to several body processes. The high levels of 18:2 in the plasma would suggest that it is required to be readily available to the body in large amounts to optimize body development and function. Another possibility relates to 18:2 being the most abundant fatty acid in the diet provided to the mares resulting in more of the fatty acid being absorbed into the blood stream. The primary theme among the saturates and mono-unsaturates was a reduction from birth, excepting 14:0, which increased in percent as time increased.

While previous research has observed increased DHA levels in the plasma of mares supplemented with an algae supplement (Adkin, 2012), this study did not observe any differences in mare plasma fatty acid levels of ALG treatment mares compared to FLAX and CON mares. The reason for this inconsistency may be due to differences in experimental design or differing supplement composition. One other observation was that mare plasma EPA levels were mostly not detectable throughout the study, regardless of treatment. Adkin (2012) also reported this finding when supplementing mares with an algal supplement. In contrast, Kouba et al. (2019) observed that when mares were supplemented with DHA and EPA (10.43 g and 8.84 g daily, respectively) for 60 days prior to parturition, they expressed higher levels of EPA and DHA in both plasma and

milk. Further, the current study is also inconsistent with findings of non-pregnant animals that have been supplemented with marine-derived omega-3's. For example, King et al. (2008) observed significantly higher EPA and DHA plasma concentrations in mares fed 681 g/day of a marine derived supplement designed to supply 10, 20 or 40 g/day of EPA and DHA depending on treatment. Similarly, Hess et al. (2012) observed elevated EPA and DHA concentrations in mares fed a marine algae supplement providing 2 g of ALA, 7.6 g of EPA, 26.6 g of DHA and 1.7 g of docosapentaenoic acid (DPA) daily. That specific study did not observe any detectable levels of EPA or DHA in mares supplemented with a flax supplement.

While previous research suggests the same trends observed in mare plasma to be observed in the milk FA analysis for various species (sows: Meers et. al, 2006; cows: Mattos et. al, 2004; women: Olafsdottir et. al, 2006), this study saw no significant differences in mare milk FA levels in regard to treatment or time. Results of treatment mare milk analysis were comparable to results of the industry mare milk analysis. The current study observed minimal levels of DHA in the mare milk and while the values suggest increased DHA in ALG treated mares, the numbers must be taken lightly due to the small sample size. Duvaux-Ponter et al. (2004), reported not observing any DHA in mare milk when supplementing with either rapeseed or linseed. More generally, Pikul and Wojtowski (2008) were not able to detect any EPA or DHA when sampling mare milk for fatty acid composition. Similarly, in humans, women supplemented with flaxseed oil did not have increased DHA levels in their breast milk (Francois et al., 2003). These reports support the theory suggesting there is limited capability for lactating mares to transfer DHA into milk. Paired with the results of the foal plasma, this suggests the

most effective avenue for supplying the foal with DHA is in utero through late gestation maternal supplementation.

With regard to neonatal health, this study observed no differences in IgG concentration of foal plasma at 12 hours of life. This is consistent with Adkin's (2012) findings that foal serum IgG content was not affected by treatment at 24-36 hours of life. While the current study did not investigate the IgG content of mare colostrum, other studies have shown that there was no difference in mare colostrum IgG content observed between mares fed marine derived algae and a basal corn diet (Kouba et. al, 2019). Previous studies had suggested that maternal supplementation of marine derived algae would result in longer gestation lengths as well as a shorter time to stand and suckle by offspring. However, this study did not observe any significant difference in treatments in any of these values for the foals. A previous study had investigated the effects of FA supplementation on rump fat thickness for mares in maintenance and had observed no differences between groups receiving corn oil and those on a control diet (Headley, et. al, 2012). The current study also observed no difference between treatments for the rump fat thickness of mares.

CHAPTER VII

CONCLUSION

Dietary supplementation of mares with marine-derived algae during late gestation alters the FA composition of the foal plasma at birth, resulting in higher concentrations of EPA and DHA. Foals that are in utero and that are suckling their dams had a transfer of these PUFA into their blood. The findings of this study paired with the previous efforts of other researchers suggests that the most efficient avenue for increasing foal DHA is through maternal supplementation during gestation, and that supplementation during the final 30 days is sufficient to see changes. Further research should investigate if beginning supplementation earlier in gestation would affect mare circulating fatty acid profiles as well as impact foal development. Furthermore, future research should investigate if continuance of supplementation to the mare through the lactation period and then to the foal in early adolescence would continue to affect foal DHA levels. While previous research has begun to investigate the beneficial effects of DHA for foals, consistent benefits remain to be determined.

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VITA

Erika Abigail Snyder

EDUCATION

Sam Houston State University, Huntsville, Texas

Master of Science in Agriculture, December 2020

Thesis: Evaluating Sources of Omega-3 Fatty Acids for Their Benefits on Late Gestation Mares and Neonatal Foals, Snyder, Bedore 2020

Cumulative GPR: 4.0

Texas A&M University, College Station, Texas

Bachelor of Science in Animal Science (Production), Cum Laude May 2018

Certificates in Equine Science and Meat Science

Cumulative GPR: 3.50

EXPERIENCE

Sam Houston State University, Huntsville, TX
Graduate Teaching and Research Assistant, August 2018- Present

- Oversee mare and foal care portion of Foaling Practicum class, foaling out over 15 mares
- Assist in the teaching of Equine Training and Stock Horse Riding classes
- Perform lab work and research duties as relating to thesis research
- Proficient in lab procedures and data management
- Understand importance of timely data collection and proper data organization
- Participate in breeding and embryo transfer procedures through partnership at Birdsong Farms with Stephen Vogelsang

Knight Land & Cattle, Midway, TX

Ranch Operations Assistant, June 2019- February 2020

- Assisted in daily ranch operations working directly under Ranch Manager
- Managed broodmare breeding appointments, medications and other related care
- Oversaw the preparation and care of horses in training
- Assisted in the production and maintenance of all ranch enterprises

Haby & Haby Farms, Brackettville, TX

Ranch Management Intern, May-August 2017

Texas A&M Agrilife Extension Service, College Station, TX

Horsemanship Instructor, April-July 2016